

GENOTYPING OF *CRTRB13'* TE POLYMORPHIC GENES FROM DIFFERENT MAIZE CULTIVARS OF MANIPUR AND TRIPURA, INDIA

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ABSTRACT

Plants have been the sources of macro- and microelements from time immemorial. The search for such variant maize having the sources of provitamin from Manipur and Tripura had been the core focus of the present work. A total of 42 cultivars of maize from different parts of Manipur and 4 cultivars from Tripura were collected and genomic DNAs were harvested from tender leaves. The template DNAs were exposed to the primers specific to *crtRB13'* TE gene. The starting from favourable allele 1 corresponding to 543bp amplicon to second important allele 2 corresponding to 296bp amplicon along with 325bp and 875bp amplicon are reporting from Manipur and Tripura for the first time. Besides these two important alleles of *crtRB1 3'* TE another third allele corresponding to 296bp, 543bp and 1880bp amplicons is reporting in the present paper. The maize with favourable alleles of 543bp amplicon having yellow kernel of Manipur and yellow and black kernel of Tripura raising an important query on relationship between yellowness and provitamin- β -carotene content. The question of whether the geographical factors have any controlled over the colour of kernel is open for the future studies.

KEYWORDS: Manipur, Maize, *CrtRB1* Gene, Cultivars, Polymorphisms

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INTRODUCTION

Plants are the directly or indirectly major sources of all food available on this vast earth although it unable to provide dietary deficiency of vitamin A. 70% of childhood death and 0.25-0.5 million blindness in worldwide is reported to affect by vitamin A malnutrition alone (<http://www.who.int/nutrition/topics/vad/en>). To fight such problems, increasing the concentration of β -carotene (pro-vitamin A) in staple crop such as maize crop through modern breeding technology is important which is more sustainable and cost efficient rather than food supplementation, fortification and dietary diversification for the human welfare (P feiffe and Mc Clafferty, 2007; Bouis and Welch). One of the important findings is the discovery of a variant genes in β -carotene biosynthetic pathway that can differ the concentration of the β -carotene (provitamin A). In the pathways, the phytoene synthase 1 (*PSY1* or *Y1*), the lycopene epsilon cyclase (*lcyE*) that increases the ratio of β to α -carotene in grain and β -carotene hydroxylase (*crtRB* which convert β -carotene to β -cryptoxanthin and Zeaxanthine in carotenoid biosynthesis) are the pivotal genes. Among the pivotal genes, Yan et al. (2010) validated that *crtRB13'* TE alone is responsible for effecting 2 to 10- fold increase in kernel β -carotene concentration in maize. The 3' TE (transposable element) polymorphism of the gene that spans the 6th exon and the 3'-UTR (untranslated region) generates three alleles, namely allele 1 (543 bp; without TE insertion), allele 2 (296 bp+875 bp; with 325 bp TE

insertion), and allele 3 (296 bp+1221 bp+1880 bp; with 1250 bp TE insertion), that were associated with altering β -carotene accumulation (Harjes et al., 2008, Vallabhaneni and Wurtzel. 2009, Yan et al., 2010). Allele 1 of the *crtRB1* gene (hereafter allele 1) is favourable and increases the level of β -carotene, whereas allele 2 and allele 3 cause unfavourable effects. Thus, identification of allele 1 (the rare gene) is highly required for provitamin A enriched maize breeding for introgression in the future bio-fortification programme. As North Eastern Hill (NEH) region of India fits to sub-tropical and temperate region with diverse geographical landscapes, agriculture ecosystem, biological diversity and their endemic and ethnic cultures, it favored rich hub of maize diversity hence can be used as source for identification and development of β -carotene rich donor for future breeding programme through marker assisted selection (MAS) (Harjes et al., 2008). However, *crtRB1* diversity in this region had not yet been studied. Therefore, present study emphasized the identification of favourable *crtRB1* allele from 42 landraces of Manipur and 4 landraces from Tripura origin using gene specific validated HYDB molecular marker. This (cultivar/landraces) can be used as a specific trait donor in efficient future breeding for development of provitamin A rich maize variety. This will hopefully fulfilled the goal of "biofortification" programme set by Harvest Plus. The present paper reports the finding of the variant maize varieties with the presence of favourable genes for provitamins starting from 299 bp and 503 bp from Manipur and Tripura of the NEH regions of India.

MATERIALS AND METHODS

Plant Materials

The seeds of maize were collected from different parts of Manipur and Tripura. In total there were 1204 study sides from Manipur and 10 study sides from Tripura. The seeds were catalogue and assigned specific codes for the study during 2013 to 2015. The seeds were planted in the institutional experimental fields and depending on the similarities in the morphological characters Kernel shape, kernel colour, kernel row arrangement and Local name, the plants were grouped into 42 distinct cultivars as a representative of Manipur and 4 cultivars from Tripura. These distinct varieties/cultivars were screened for the *crtRB1* genes.

DNA Extraction

The seeds were planted in laboratory to obtain the young leaves in 15 days and the leaves were cut off to extract genomic DNA using CTAB method of Sanghai Maharoof et al. (1984) with slight modification.

PCR

Polymerase chain reaction for the marker specific to the *crtRB1* 3' TE was performed using the following set of primers: *crtRB1*-3' TE-F: ACACCACATGGACAAGTTCG, *crtRB1*-3' TE-R1: ACACTCTGGCCCATGAACAC, and *crtRB1*-3' TE-R2: CAGCAATACAGGGGACCAG. For the PCR cycle, the procedure given by Yan et al. (2010) was followed. Primers F and R2 amplify the intact *crtRB1* 3' TE region and produce a single amplicon (allele 1), whereas primer R1, which is specific to the TE insertion, amplifies the insertion region within the *crtRB1* 3' TE gene and generates more than one fragment, allele 2 and allele 3. The amplified fragments were resolved using agarose gel electrophoresis (1.5% agarose) and scored for the presence of allele polymorphism through Bio-rad gel documentation unit.

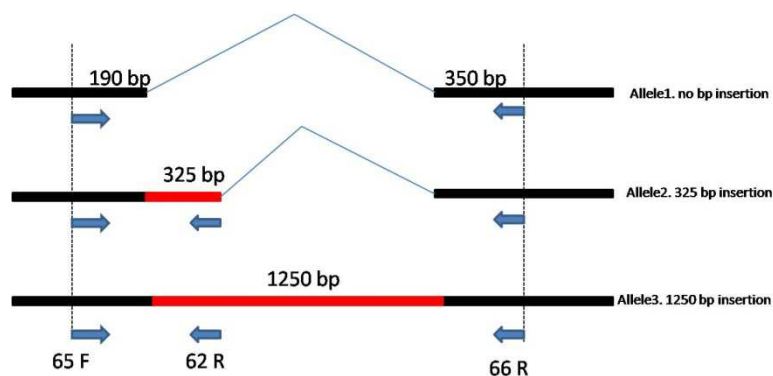


Figure 1

Figure 1 Schematic of 3' TE polymorphism of *crtRB1* gene (Modified from Yan et al., 2010). The genetic variation due to insertions at “3' TE” (a polymorphic site, spanning the 6th exon and the 3' UTR and located between the amino acids 190aa and 350aa, at the 3' end) of the *crtRB1* gene creates three alleles that are associated with variation in β -carotene accumulation in the kernel. Allele 1 is without insertion and a PCR of this allele with the indicated primer set results in an amplicon of size 543bp. Allele 2 has a 325bp insertion and a PCR of this allele with the indicated primer sets results in an amplicon of size 296 + 875bp. Allele 3 has a 1250bp insertion and a PCR of this allele with the indicated primer sets results in an amplicon of size 296+1221+1800. (However, the largest amplicon (1800 bp) amplified by the primers 65F and 66R was usually weak or not amplified.) Of the three alleles of 3' TE, allele 1 is known as a favorable allele, for it is associated with the enhancement of β -carotene concentration in the maize grain.

(Selvi et al., 2014, Yan et al., 2010)

RESULTS AND DISCUSSIONS

The first step in breeding maize for enhanced β -carotene involves an assessment of genetic variability existing in adapted germplasm for developing an appropriate breeding strategy (Vignesh et al., 2012). One of the important findings regarding the provitamin sources from maize plants is the discovery of a variant genes in β -carotene biosynthetic pathway that can differ the concentration of the β -carotene (provitamin A). In the pathways, the phytoene synthase 1 (*PSY1* or *Y1*), the lycopene epsilon cyclase (*lcyE*) that increases the ratio of β to α -carotene in grain and β -carotene hydroxylase (*crtRB* which convert β -carotene to β -cryptoxanthin and Zeaxanthine in carotenoid biosynthesis) are the pivotal genes. Among the pivotal genes, Yan et al. (2010) validated that *crtRB13' TE* alone is responsible for effecting 2 to 10- fold increase in kernel β -carotene concentration in maize. The 3' TE (transposable element) polymorphism of the gene that spans the 6th exon and the 3'-UTR (untranslated region) generates three alleles, namely allele 1 (543 bp; without TE insertion), allele 2 (296 bp+875 bp; with 325 bp TE insertion), and allele 3 (296 bp+1221 bp+1880 bp; with 1250 bp TE insertion), that were associated with altering β -carotene accumulation (Harjes et al., 2008, Vallabhaneni and Wurtzel. 2009, Yan et al., 2010). Co-dominant marker for the 3' TE region of the *crtRB1* gene was identified using polymerase chain reaction (PCR) and rapidly improving β -carotene concentration in maize kernels through MAS (Harjes et al., 2008).

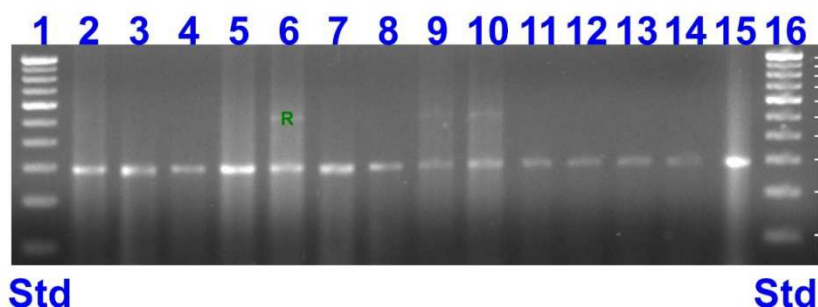


Figure 2

Figure 2: Allelic variation at *crtRB1* 3'TE gene among the Manipur and Tripura genotypes. Lane 1 – 100bp; Lane 6- CAUCH 87 (landraces from Manipur *crtRB1* 3'TE favourable allele: 543 bp); Lane 9- CAUCH 1204, Lane 10 CAUCH 1206 (landraces from Tripura *crtRB1* 3'TE favourable allele: 543 bp); Lane 2- CAUCH 39, Lane 3- CAUCH 173, Lane 4- CAUCH 118, Lane 5- CAUCH 38, Lane 7- CAUCH 50, Lane 8- CAUCH 28, Lane 11- CAUCH 36, Lane 12- CAUCH 71, Lane 13- CAUCH 1004, Lane 14- CAUCH 95 and Lane 15- CAUCH 93 Landraces with unfavourable allele (296 bp); Lane 16: 100 bp DNA ladder.

Among the 46 morphological distinct cultivars only 3 genotypes viz; CAUCH 87 (Manipur landrace), CAUCH 1204 (Tripura Landrace) and CAUCH 1206 (Tripura Landrace) were found to present allele 1 *i.e.* 543 bp while other genotypes were found to present allele 2 *i.e.* 296 bp. Presence of allele 1 in CAUCH 87, CAUCH 1204 and CAUCH 1206 is due to no insertion of any base pair in the 3'TE region of *crtRB1* gene that responsible for higher accumulation of β -carotene in maize kernel. This rare favourable genetic constitution in *crt RB1* gene might be possibly arises from natural microclimatic conditions and genetic heterogeneity of studied region (NEH). And unfavourable allele 2, common type that shows 296 bp indicates insertion of 325 bp in 3'TE region of the *crtRB1* that lower accumulation of β -carotene in maize. Therefore the 3 genotypes viz; CAUCH 87, CAUCH 1204 and CAUCH 1206 possessing allele 1 could be used as donor in breeding programme to elite adapted lines for improvement of provitamin A in maize to meet the dietary requirement.

CONCLUSIONS

The NEH region of India as included in Hottest biodiversity as Indo-Burma hotspots (Myers et al., 2000) have the variant maize varieties having the favourable genes for the biofortification of vitamin A through introgression of the land races CAUCH 87, CAUCH 1204 and CAUCH 1206 in future breeding tasks.

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REFERENCES

1. Harjes, C.E., T.R. Rocheford, L. Bai, T.P. Brutnell, C.B. Kandianis, S.G. Sowinski, A.E. Stapleton, R. Vallabhaneni, M. Williams, E.T. Wurtzel, J. Yan, and E.S. Buckler. 2008. Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science* 319:330–333.
2. Vallabhaneni, R., and E.T. Wurtzel. 2009. Timing and biosynthetic potential for carotenoid accumulation in genetically diverse germplasm of maize. *Plant Physiol.* 150:562–572.

3. Yan J., Kandianis B. C., Harjes E. C., Bai L., Kim H. E., Yang X., Skinner D. J., Fu Z., Mitchell S., Li Q., Fernandez G. S. M., Zaharoeva M., Babu R., Fu Y., Palacios N., Li J., DellaPenna D., Brutnell T., Buckler S. E., Warburton L. M. and Rocheford T. 2010. Rare genetic variation at *Zea mays crtRB1* increases β -carotene in maize grain. *Nature Genetics*, **42**: 322-327.
4. Saghai-Marooof M.A., Soliman K.M., Jorgensen R.A. and Allard R.W.(1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. USA* **81**:8014-8018.
5. Thirusendura D. Selvi, N. Senthil, A. Yuvaraj, A. John Joel, A. Mahalingam, P. Nagarajan, S. Vellaikumar, P. Srimathi, M. Raveendran, and T. Nepolean. **Assessment of crtRB1 Polymorphism Associated with Increased β -Carotene Content in Maize (*Zea mays* L.) Seeds** 2014, *Food Biotechnology*, **28**:41–49.
6. Pfeiffer W. H. and McClafferty B. 2007. HarvestPlus: Breeding crops for better nutrition. *Crop Sci.*, **47**: S88- S105.
7. Bouis H. E. and Welch R. M. 2010. Biofortification-asustainable agricultural strategy for reducing micronutrient malnutrition in the global South. *Crop Sci.*, **50**: S20-S32.

